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An N-acetylglucosamine transporter required for arbuscular mycorrhizal symbioses in rice and maize

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Abstract

Most terrestrial plants, including crops, engage in beneficial interactions with arbuscular mycorrhizal fungi. Vital to the association is mutual recognition involving the release of diffusible signals into the rhizosphere. Previously, we identified the maize *no perception 1* (*nope1*) mutant to be defective in early signaling. Here, we report cloning of *ZmNOPE1* on the basis of synteny with rice. *NOPE1* encodes a functional homolog of the *Candida albicans* N-acetylglucosamine (GlcNAc) transporter *NGT1*, and represents the first plasma membrane GlcNAc transporter identified from plants. In *C. albicans,* exposure to GlcNAc activates cell signaling and virulence. Similarly, in *Rhizophagus irregularis* treatment with rice wild type but not *nope1* root exudates induced transcriptome changes associated with signaling function, suggesting a requirement of NOPE1 function for presymbiotic fungal reprogramming.

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Author contribution: M.N., R.S., N.G., E.M., J.B.K., T.P.B. and U.P. designed the experiments. M.N., R.S., S.N., B.B., C.K., A.S., G.A., K.R.A., A.R., C.G., and C.R., performed the experiments. C.R. performed bioinformatics and statistical analyses of the RNAseq data. M.N., R.S., C.R., E.M., J.B.K. and U.P. wrote the manuscript.

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Introduction

Arbuscular Mycorrhizal (AM) symbiosis is a mutually beneficial relationship between plants and fungi in which plant roots exchange photoassimilates for fungus-delivered soil minerals. The resulting interaction may profoundly influence plant performance, in both wild and cultivated systems. For the symbiosis to begin, plant roots and AM fungi (AMF) exchange signals via secretion of diffusible compounds[1], including fungal chitin-based molecules (reviewed in[2,3]) detected by Lysine Motif (LysM) containing plasma membrane receptor-like kinases[4,5]. Central to the perception of AMF in rice is the α/β hydrolase DWARF14 LIKE (D14L) and the F-box protein DWARF3 (D3)[6], although, in this instance, the signal molecules remain uncharacterized.

A number of plant-derived factors are known to stimulate morphological changes in AMF, promoting fungal-host encounters[1], including flavonoids that enhance hyphal tip elongation[7], 2-hydroxid fatty acids (2-OH-FA) that trigger hyphal branching[8], and strigolactones (SL) that induce changes in fungal metabolism, coupled with profuse hyphal ramification[9,10,11]. Despite their pre-symbiotic effect on AMF growth, plant SL and flavonoid biosynthetic mutants are still partially or fully colonized[12,13]. Once the fungus has reached the plant's surface, cutin monomers induce hyphopodium differentiation, the anchoring structure for entry of AMF into the root epidermal cell layer[14]. As the fungal genome lacks genes for the *de novo* biosynthesis of certain fatty acids[15], cutin may have an additional nutritional role.

To better understand signalling during symbiotic establishment, we analyzed the maize *no perception1* (*nope1*) mutant, which does not form arbuscular mycorrhizal symbioses[16]. We identified *NOPE1* to encode an N-acetylglucosamine (GlcNAc) transporter, a function not described previously in plants, but characterized in fungi[17]. Notably, GlcNAc has been shown to stimulate the fungal pathogen *Candida albicans* to undergo morphological changes and increase expression of virulence genes that promote pathogenic interactions with the host[18]. Our analyses provide the first evidence that a previously unknown plant GlcNAc transporter plays a role in the initiation of root colonization by AMF.

Materials & Methods

Plant and fungal material

Oryza sativa ssp. japonica cv. Donjing, *Zea mays* inbred W22 and *Arabidopsis thaliana* ecotype Col-0 were used throughout the study. *R. irregularis* and *G. rosea* spores were either axenically produced[5] or purchased (Premiertech, Rivière-du-Loup, Canada; Agronutrition, Toulouse, France). *Piriformospora indica, Magnaporthe oryzae* and *Candida albicans* strains were propagated as previously described[1,4,7].

Identification of rice *NOPE1*

The maize *nope1* locus was mapped to a ~10Mb interval on chromosome 10, defined by the markers UMC1336 (86.3Mb) and Phi071 (93.7Mb; Fig. S1A). The syntenic region on rice Chromosome 4S contains AM-inducible *LOC_Os04g01520*[8] (Fig. S1A). According to full cDNA and EST information *LOC_Os04g01520* consists of two exons and one intron (Fig.

S1B), producing an ORF of 1404 bp. The 5' and 3' RACE PCR analysis indicated a transcriptional start point at −70 bp and a 184 bp 3'UTR sequence. The gene product of *LOC_Os04g01520* consists of 476 residues and has a predicted molecular weight of 50.34 kD.

Identification of *OsNOPE1* mutant

Two rice lines, *4A-01057* and *3A-02512,* were identified from public mutant collections[19] with T-DNA insertions 158 bp downstream of ATG within the first exon, and 22 bp upstream of the 3' intron splice-junction, respectively (Fig. S1B). RT-PCR based analysis of *LOC_Os04g01520* mRNA levels detected no or wild type levels in *4A-01057* and *3A-02512*, respectively (Fig. S1C). Amplicon sequencing from line *4A-01057* confirmed the predicted mutation and revealed the additional presence of ~800 bp of the backbone vector (pGA2517, Fig. S1B).

Genetic complementation of *Osnope1* mutant

The genomic region of *LOC_Os04g01520* including 1.5kb of promoter sequence were amplified and cloned into pGEM-T Easy (Promega, Dübendorf, Switzerland) to generate pRS909. The *NOS* terminator sequence was amplified with primers RS976 and RS977 (Supplemental Table S4) and inserted into the *Nde*I and *Sac*I (Promega, Dübendorf, Switzerland) site of pRS909 to generate pRS934. Finally, pRS934 was digested with *Bgl*II and *Sac*I (Promega, Dübendorf, Switzerland) and the resulting segment gel-isolated and cloned into binary vector pTF101.1[14] to generate pRS936. Stable rice transformation was performed as previously reported[17].

Rice GlcNAc transport assays

Rice seedlings were grown in ½ MS medium (described in supplementary information) and transferred to equilibration solution, containing 10 mM MES-KOH at pH 6; 1 mM $CaCl_2$ (ES) for 1h. Four seedlings were pooled per sample and 3 replicates per genotype were used. Seedlings were moved to incubation solution (IS), consisting of ES; 3kBq/ml [^{3}H]GlcNAc (American Radiolabeled Chemicals, Saint Louis, MO). GlcNAc concentration was adjusted to 100 μM with cold GlcNAc (Sigma-Aldrich, Dorset, UK). For uptake experiments, roots were washed twice for 30 s with ice-cold washing solution (WS): ES; 100 μM GlcNAc (cold). For efflux experiments, roots were left for 2 h in IS, washed twice for 30 s with WS and transferred to a solution either lacking GlcNAc: ES, or supplemented with 50× GlcNAc: ES; 5 mM GlcNAc and incubated as indicated in results. To quantify [^{3}H]GlcNAc content, roots were excised and placed in vials containing 3 ml of 0.1 N HCl for 1 h. Of the extracted fluid 1.6 ml was collected and [^{3}H]GlcNAc was quantified by scintillation counting.

Genetic complementation of *Candida albicans*

Growth of *C. albicans* strains was examined by spotting a 10-fold cell dilution onto agar medium containing Yeast Nitrogen Base minimal medium and 50 mM of GlcNAc, glucose, or galactose (ThermoFisher Scientific, Grand Island, USA). Plates were incubated at 30°C for 2 days and then photographed. Induction of hyphal morphogenesis was examined by growing cells overnight at 37°C in minimal medium containing glucose, then resuspending

them at 10^6 cells/ml in medium containing either 50 mM glucose or 50 mM GlcNAc and incubation for 2 h before documentation. The results were reproducible with different colonies and isolates obtained from two independent transformations.

Root exudate and GlcNAc treatment of *Rhizophagus irregularis*

Sampling of rice root exudates included five six-week old plants from sand-cultivated wild type and *Osnope1* genotypes. The roots were well-washed, transferred to individual 1 l Erlenmeyer flasks containing ~750 ml of ½ HL (50 μM KH_2PO_4) solution and incubated with gentle agitation. After 3 days, exudates were harvested, sterilized using 0.2 μm filters (Sartorius Epsum, Surrey, UK) and immediately used. Groups of 80'000 spores of *R. irregularis* per replicate were germinated at 2% [CO_2] and 30° C for 7 days in 8 ml of *R. irregularis* minimal medium[5]. Pre-germination solutions were replaced with 8 ml of wild type (Donjing) or *Osnope1* rice exudates, or with 8 ml of 50 mM GlcNAc. Fungal material was collected at 0, 1 h, 24 h, and 7 d post treatment.

RNAseq sequencing and data analysis

Fungal transcriptome analysis was performed on three independently grown replicates. Please see supplementary information for detailed description of nucleic acid handling and library preparation. RNAseq sequencing involved the Illumina HiSeq2000 (Illumina Inc., San Diego, USA) using a 2 × 100 bp pair-end strategy with Illumina TruSeq SBS sequencing kits v3 (PN FC-401-3001, HiSeq2000). Sequencing was performed at the GeT (Genome & Transcriptome Core facilities, Toulouse, France, http://get.genotoul.fr/). RNAseq reads have been released at NCBI Gene Expression Omnibus (accession n° GSE65595).

Reads were mapped to the Gloin1 assembly[21] to define transcript accumulation patterns by RNA-seq functionality of the CLC Genomic Workbench suite. Significantly differentially expressed genes were identified by calculating RPKM (Reads Per Kilobase of exon per Million fragments mapped) and proportion-based test statistics[22] with a False Discovery Rate FDR[23] correction for multiple testing (settings: minimum mapped read length fraction = 0.95; minimum similarity = 0.98). According to CLC recommendations, genes were significantly upregulated when meeting the requirements of "total difference reads mapped" >10, RPKM fold change >2 and FDR corrected $p < 0.05$. Gene set enrichment involved the unconditional GOstats test of Falcon and Gentleman[24] based on Gloin1 annotation available at http://genome.jgi.doe.gov/cgi-bin/ToGo?accession=all&species=Gloin1. The "p-value" corresponded to the tail probability of the hyper geometric distribution.

Data availability

R. irregularis RNAseq reads have been released at Gene Expression Omnibus (accession n° GSE65595). All other datasets (Supplemental Tables S1–S3) are included in this published article. *C. albicans* strains used in this study are available from J.B.K. (james.konopka@stonybrook.edu). All other data that support the findings of this study are available from the corresponding author upon request.

Results

Cloning of maize *ZmNope1* on the basis of synteny with rice

The maize *Zmnope1* mutant is unable to establish AM symbioses[16]. Limited physical interaction of *Zmnope1* with AMF suggested a failure in pre-symbiotic signal exchange. As genetic mapping had linked the *Nope1* locus to the marker UMC1336 on chromosome 10, we searched for rice candidate genes exhibiting a transcriptional response to mycorrhizal root colonization located in the region syntenic to maize *nope1*[19, 20], identifying the gene *LOC_Os04g01520*[19] (Fig. S1A). To investigate a potential role in AM symbiosis, rice plants segregating for a T-DNA insertion in *LOC_Os04g01520* (*4A-01057*; Fig. S1B, C) were cocultivated with *R. irregularis*. A significant reduction in fungal root colonization was observed in plants homozygous for the insertion ($p \leq 0.05$ for all structures tested; Fig. 1A). On the surface of mutant but not wild type roots, the fungus formed aberrant hyphopodia (Fig. 1B–C). Closer inspection of aberrant hyphopodia revealed multiple unsuccessful penetration attempts (Fig. 1D, arrowheads) and extensive hyphal septation, a sign of fungal stress (Fig. 1C, arrows). Infrequently, the fungus did succeed in invading the root cortex, and produced arbuscules that were of wild type morphology (Fig. 1E–F). To confirm that the phenotype was indeed linked to disruption of *LOC_Os04g01520*, we reintroduced a wild type copy of the gene under the native promoter, and observed a restoration of wild-type levels of AM fungal colonization (Fig. 1A, G; Fig. S2). In addition, transcript levels of previously described AM marker genes[21] were reduced in *Osnope1* homozygous plants, but restored to wild type levels in complemented lines (Fig. S4). The quantitative and qualitative phenotype of the rice insertion mutant was equivalent to that of the reported maize *Zmnope1* mutant[16] and, consequently, *LOC_Os04g01520* was designated *OsNOPE1*, and the insertion *4A-01057, Osnope1-1*. To investigate whether NOPE1 is required for susceptibility to different fungi known to invade rice roots[21,22], wild type and *Osnope1* mutants were inoculated with *Piriformospora indica* and *Magnaporthe oryzae*. Both fungi invaded mutant and wild type root tissue equally well (Fig. S3), suggesting that NOPE1 might be required specifically for interaction with AMF.

Mutation of maize *ZmNope1* results in a phenotype equivalent to that of *Osnope1-1*

The maize *nope1* mutant arose in a population with high levels of *Mutator* transposon activity[16], and suppression of the phenotype of the original allele prevented further direct characterization of the maize *nope1* mutation. Instead, we undertook a reverse genetics approach to verify the role of the maize homologue of *OsNOPE1* in AM symbiosis. The maize genome contains a single gene, *GRMZM2G176737,* showing high similarity to *OsNOPE1* (BLASTP; score=488, ID=83%, e-value=$8.2\times e^{-61}$), located on Chromosome 10 near to the mapped position of the *nope1* mutation (Fig. S1A). A *Dissociation* (*Ds)* transposon linked to *GRMZM2G176737* was identified and re-mobilized[23,24], generating a novel insertion (*Zmnope1-1*) within the first exon, that resulted in an absence of transcript accumulation (Fig. S5A, B). Quantification of intraradical fungal structures in *Zmnope1-1* homozygous plants revealed significantly lower fungal colonization ($p < 0.05$ for all structures tested) compared with wild type plants (Fig. 1H). Despite fungal proliferation on the root surface, hyphopodia were malformed and failed to penetrate (Fig. 1H–J). In addition, transcript levels of the maize homologues of the rice AM marker genes *OsAM3*[21]

and *OsPT11*[25], *GRMZM2G135244* (*ZmAM3*) and *GRMZM5G881088* (*ZmPT6*)[26], were lower in inoculated roots homozygous for the transposon insertion as compared to hemizygous and wild-type siblings (Fig. S5C). The syntenic genetic location and equivalent loss-of-function phenotypes of rice and maize *NOPE1* genes strongly suggests that they are orthologous, and that this maize gene was mutated in the previously reported *nope1* mutant[16].

NOPE1 belongs to the Major Facilitator Superfamily and is found in all land plants

Analysis of the predicted NOPE1 protein (http://phobius.binf.ku.dk/; http://smart.embl-heidelberg.de/) identified 12 transmembrane domains, no signal peptide and a domain of unknown function (DUF895) between amino acids 49 and 181 (Fig. S6). NOPE1 was identified as member of the Major Facilitator Superfamily (MFS, Pfam e-value $4.9e^{-13}$), suggesting a role in transport of small molecules across membranes. BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) indicated genes encoding NOPE1 to be present in the genomes of all land plants for which data was available, including non-mycorrhizal plant species (Fig. S6). The genome of *Medicago truncatula* contains two *NOPE1* orthologs; *Medtr3g093270* and *Medtr3g093290;* the gene products share 63% identity (79% similarity) and 64% identity (81% similarity) with OsNOPE1, respectively, providing an explanation for the absence of *NOPE1*-associated AM phenotypes from forward genetic screens in legumes. The genome of *Arabidopsis thaliana*, a non-host for AMF, also contains two *NOPE1* orthologs (*At1g18000* and *At1g18010*), expressed constitutively throughout the plant (https://bar.utoronto.ca/eplant/[27]). To determine the possible role of NOPE1 in *Arabidopsis*, RNAi-based silencing was used to down-regulate both genes simultaneously (Fig. S7), but we did not detect any phenotypic effect under standard conditions, with respect to germination, morphology, flowering time or seed set.

Rice NOPE1 mediates N-acetylglucosamine transport in *Candida albicans*

To investigate the mechanism by which NOPE1 influences AM symbioses, we sought to identify putative homologs with functional annotation. As no land plant orthologs had been characterized, we focused on the protein Ngt1 of the human pathogenic fungus *Candida albicans*. Ngt1 shows 41% identity at the protein level with OsNOPE1, and the two proteins are reciprocal best hits in a BLAST search. Ngt1 mediates N-acetylglucosamine (GlcNAc) transport across the plasma membrane of *C. albicans,* enabling growth when GlcNAc is the sole carbon source[28]. A key feature of *C. albicans* virulence is the ability to reversibly shift from isotropic budding to polarized filamentous growth in response to environmental signals[29]. Amongst the stimuli triggering this morphological switch is GlcNAc[30]. Deletion of the *NGT1* gene impairs GlcNAc uptake, preventing cells from switching morphology and from proliferating on GlcNAc-containing medium[17,31]. To address whether OsNOPE1 is a functional GlcNAc transporter, a *C. albicans* codon-optimized version of the rice *OsNOPE1,* driven by the native fungal *NGT1* promoter, was transformed into the *C. albicans* mutant *ngt1*Δ. Remarkably, introduction of rice *OsNOPE1* restored growth and induction of filamentous hyphal differentiation on GlcNAc medium (Fig. 2A–C), indicating functional conservation of the protein across plant and fungal kingdoms.

To investigate substrate specificity of OsNOPE1, competition assays were performed in which an excess of cold hexoses was provided together with [^{3}H]GlcNAc. Control studies showed that addition of a 2-fold excess of cold GlcNAc led to a partial decline in the uptake of radioactive GlcNAc but nearly complete inhibition at 20-fold excess cold GlcNAc (Fig. S8). OsNOPE1 showed strong specificity for transporting GlcNAc, since a 200-fold excess of glucosamine, dextrose, fructose or galactose did not significantly impact on the amount of [^{3}H]GlcNAc transported into the cells. A 200-fold excess of N-acetylmannosamine partially competed with [^{3}H]GlcNAc ($p < 0.01$ by non-parametric one-way ANOVA), suggesting that the N-acetyl moiety may be important for substrate specificity. Overall, these results demonstrated that OsNOPE1 exhibits a high specificity for transporting GlcNAc, similar to *C. albicans* Ngt1.

NOPE1 mediates GlcNAc transport in plants

Heterologous expression in *C. albicans* suggested that NOPE1 may mediate GlcNAc transport across plasma membranes also in plants. We characterized subcellular localization of the *Arabidopsis* NOPE1 protein At1g1800 by stable, constitutive expression of an in-frame fusion to yellow fluorescent protein (YFP). Three independent transgenic lines showed a reproducible and clear signal consistent with plasma membrane localization (Figure 3A).

To explore the capacity of OsNOPE1 to transport GlcNAc *in planta*, we measured [^{3}H]GlcNAc root uptake in wild type, *Osnope1* and complemented mutant rice seedlings. The rate of [^{3}H]GlcNAc was significantly ($p < 0.05$) reduced in *Osnope1* mutants compared with wild type or complemented plants (WT: 2.37 pmol h-1; C4: 2.00 pmol h-1; *Osnope1*: 0.624 pmol h-1; Fig. 3B). To test if NOPE1 supported transport of GlcNAc across cell membranes of non-mycorrhizal plants, we measured the [^{3}H]GlcNAc uptake in protoplasts derived from the *Arabidopsis At1g1800* overexpression line *At4731y-4* and compared this uptake to that of the RNAi-silencing line *AtMNC58*. Uptake of [^{3}H]GlcNAc was significantly ($p < 0.01$) higher in the overexpression line *At4731y-4* compared to the RNAi-silenced line *AtMNC58* (Fig. S10). Taken together, NOPE1 mediated GlcNAc uptake in whole roots and leaf protoplasts of mycorrhizal and non-mycorrhizal plant species, respectively.

We quantified GlcNAc efflux in the rice seedling system by first loading roots of wild type rice seedlings with 100 μM [^{3}H]GlcNAc and then transferring them to a solution containing either unlabeled GlcNAc at 50× concentration or no GlcNAc. Monitoring the levels of root-retained [^{3}H]GlcNAc revealed that in both cases GlcNAc was released from the roots, but that significantly less ($p < 0.05$) remained in the roots at shorter times when the external medium contained no GlcNAc (2.21 ± 0.10 [^{3}H]GlcNAc/plant) as opposed to high GlcNAc medium (3.30 ± 0.36 [^{3}H]GlcNAc/plant, Fig. 3C), indicating that substrate availability at the external side partially inhibited efflux.

Distinct transcriptional responses of *R. irregularis* to rice wild type and *nope1* root exudates

To characterize fungal responses to root-released compounds, RNAseq analysis was performed on pre-germinated *R. irregularis* spores exposed to either rice wild type or *Osnope1* root exudates for 1h, 24h and 7 days. Interestingly, exposure to wild-type and *Osnope1* exudates led to distinct expression profiles across the three time points. Treatment with wild-type exudates gradually enhanced the number of induced transcripts from 92 to 283 to 901 at 1 h, 24 h and 7 d (Fig. 4A). In contrast, treatment with *Osnope1* root exudates did not result in a significant change in transcript accumulation patterns until 24 h, and then fewer genes were responsive (343 and 256 induced genes at 24 h and 7 d, respectively; Fig. 4A). In the wild type treatment, d.e. genes at 1 h were enriched for the gene ontology (GO) terms protein kinase and ATPase activity (Fig. 4B) suggesting an early induction of fungal signaling activities. At 24 h, both wild type and mutant exudates led to a significant change in the fungal transcriptome, however GO analysis suggested that while the fungus switched to an elevated oxidative status in response to wild-type exudates, mutant exudates induced stress responses. By 7 days, the stress response signature remained for the mutant exudate treatment, while d.e. genes with wild-type exudate treatment were enriched with GO terms corresponding to a higher energetic and metabolic status (Fig. 4B). Collectively, these data are consistent with an early and transient activation of fungal signaling, followed by the activation of genes involved in primary metabolism in wild type, but not *Osnope1,* root exudate treated fungus. We assayed also fungal growth responses, treating *Gigaspora rosea* with exudates from wild type and *Osnope1*, but no difference was observed in the number of hyphal apices and overall hyphal growth between treatments (Fig. S10), assigning the NOPE1 associated compound to a different functional class than the previously characterized SLs, 2-OH fatty acids or flavonoids, all of which trigger specific hyphal growth patterns.

RNAseq results were validated by qRT-PCR analysis (Fig. S11). The *R. irregularis NGT1* homologue MIX9501_16_76,[38] displayed a basal expression level but was not induced in response to treatment with either exudates or by GlcNAc treatments (Fig. S12). The specific fungal transcriptional response to rice wild type root exudates is consistent with the hypothesis that the NOPE1-mediated release of GlcNAc is required by *R. irregularis* for adequate reprogramming prior to host colonization. However, application of GlcNAc to *R. irregularis*-inoculated *Osnope1* mutant plants for seven weeks at 1 mM, 10 mM or 100 mM GlcNAc did not complement the mutant phenotype. This may be due to application of GlcNAc outside the biologically active concentration, or that either the development of a GlcNAc gradient or efflux of a GlcNAc-conjugate might be necessary for stimulating the fungus. We examined whether the presence of total wild type exudates would restore AM colonization of the mutant when cocultivated within the same container, and, indeed, *Osnope1* was fully colonized when grown together with wild type but not with mutant ‘donor’ plants (Fig. S13).

Discussion

NOPE1 is a plasma membrane GlcNAc transporter required for the initiation of AM symbiosis in both rice and maize. Current knowledge of plant-derived signals in AM symbiosis is largely limited to the stimulatory effects of SLs on fungal metabolism and development, although it has been anticipated that additionally secreted bioactive molecules are necessary for establishment of the symbiosis ([34], for review see[1]). Wild-type, but not *Osnope1*, root exudates induced signaling-associated transcriptional responses in *R. irregularis* within the first hour of treatment, suggesting a priming of the fungus prior to establishment of AM symbiosis. The restoration of normal levels of colonization in *Osnope1* by co-cultivation with wild-type plants further indicated bioactive molecules, essential for successful establishment AM symbiosis, to be absent from *Osnope1* exudates. We hypothesize that a NOPE1 transported factor or factors act non-redundantly with other previously characterized plant-derived signals to prime the fungus, prior to establishment of AM symbiosis (see model Fig. S14).

NOPE1 mediates efficient GlcNAc import in *C. albicans*, rice and Arabidopsis. GlcNAc is commonly found in microbial environments, as a building block of fungal and bacterial cell walls, or as a signaling molecule, such as in rhizobial nod-factors, and GlcNAc transporter activities have been characterized in a number of microbial systems. NOPE1, however, represents, to our knowledge, the first plasma membrane GlcNAc transporter to be described in plants. Wild-type rice roots were shown to acquire and release GlcNAc, with uptake clearly dependent on NOPE1, although the role of NOPE1 in GlcNAc efflux was less clear. It has been shown previously that GlcNAc monomers are abundant in the leaves of *Arabidopsis*[35]. Although not directly quantified in roots, blocking the biosynthesis of the activated substrate for GlcNAc transfer, UDP-GlcNAc, in rice impaired cell expansion in the root elongation zone, leading to a short root phenotype, indicating an important role for GlcNAc metabolism in normal root development[36].

Host-secreted GlcNAc is known to act as a potent signaling molecule for a number of microbial organisms, including the facultative human pathogenic fungus *C. albicans*[18]. Exposure to GlcNAc leads to the induction of invasive hyphal growth and the expression of virulence genes such as the adhesins that facilitate attachment to host cells (for review see[28]). Also, the thermally dimorphic pathogenic fungi *Histoplasma capsulatum* and *Blastomyces dermatitidis* respond to treatment with GlcNAc by a similar yeast-to-filament switch[37]. In these facultative human pathogens GlcNAc additionally functions as a source of sugar. The utilization of plant-derived GlcNAc as a substrate was recently reported for the plant-pathogenic bacteria *Xanthomonas campestris* pv. *campestris* while infecting leaves of *Brassica oleracea*[38]. The closely related vector-borne phytopathogenic bacterium *Xylella fastidiosa* enzymatically digests GlcNAc polymers (chitin) available in the foregut of the insect vector, also using the acquired GlcNAc as a nutrient source[39]. Remarkably, the effects of host-provided GlcNAc extend to the mutualistic association between bioluminescent squid and vibrio, where host-derived GlcNAc acts as a regulator of shifting the bacterial metabolism to provide optimal symbiont services to the host[40]. Determining the chemical identity of the bioactive molecule associated with NOPE1 remains an exciting future

challenge, and whether NOPE1 contributes to microbe perception or influences root exudate composition requires further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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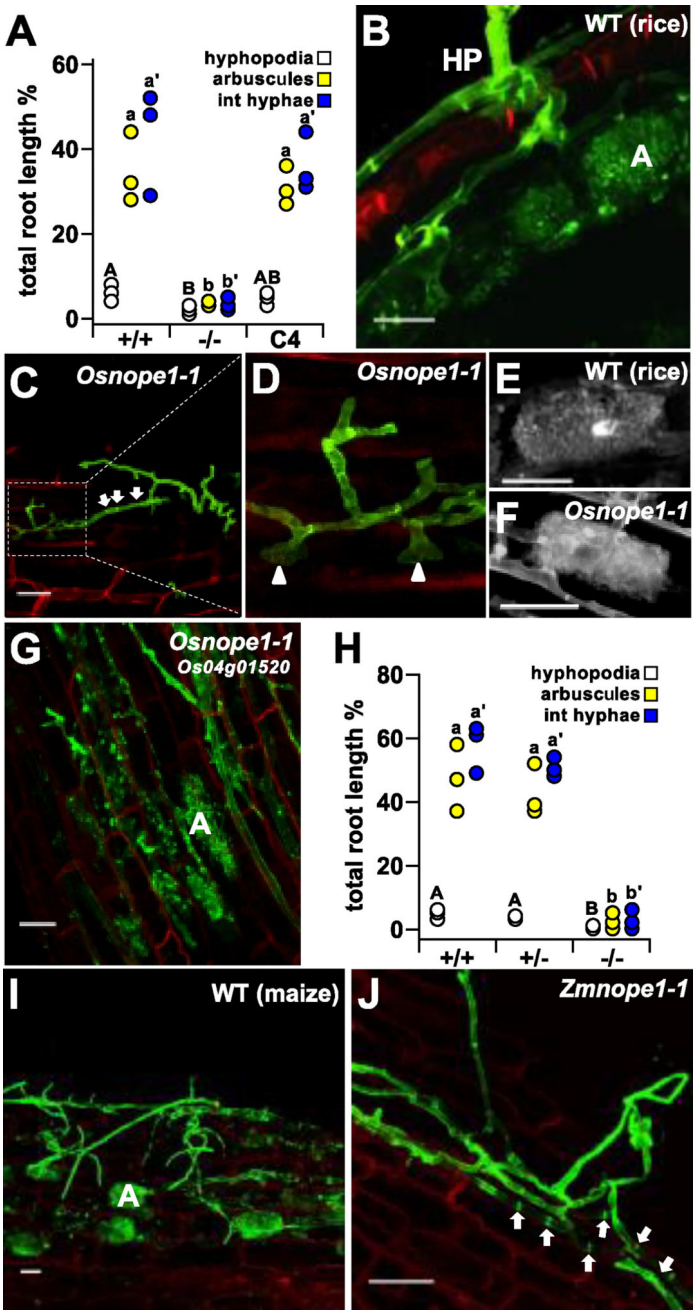

Figure 1. Mutation of the orthologous genes *Os04g01520* and *GRMZM2G176737* disrupted coloniztion by *R. irregularis* in rice and maize, respectively

A, Percentage root length colonization in rice plants (n = 3) segregating *Osnope1* (+/+, wild type, –/– homozygous *Osnope1,* C complemented line C4) at 6 weeks post infection (wpi). Means groups assigned for each fungal structure indicated by letters (adj. P < 0.05). **B–G,** WGA-staining of fungal structures and propidium-iodide counterstained plant cell walls of rice roots inoculated with *R. irregularis* at 6 wpi as examined by laser scanning confocal microscopy. **B**, Hyphopodium (HP) and arbuscule differentiation in a wild type (WT) root **C,** Misshapen and highly septate (arrows) hyphopodial hypha on the surface of the root of a

Osnope1 homozygote. **D,** Detail of a hyphopodia on a *Osnope1* homozygous plant, showing several aborted penetration attempts (arrowheads). Morphologically equivalent arbuscules formed in the roots of wild type (**E**) and *Osnope1* homozygous (**F**) plants. **G**, Arbuscules formed in root cortical cells of the complemented line C4. **H**, Percentage *R. irregularis* root length colonization of maize plants segregating *Zmnope1* (+/+, wild type, +/−, heterozygote, −/− homozygous mutant) at 6 wpi. Represented as **A**. **I** and **J,** WGA-staining of fungal structures and propidium-iodide counterstained plant cell walls of maize roots inoculated with *R. irregularis* at 6 wpi, as examined by laser scanning confocal microscopy. On wild type (**I**) roots the fungus develops normal hyphopodia and extensively colonizes the root forming frequent arbuscules. **J**, Misshapen hyphopodium on the roots of a *nope1-1* homozygous mutant showing mutiple septa (arrows) and absence of root pentration. HP, hyphopodium; A, arbuscule. Scale bar = 50μm.

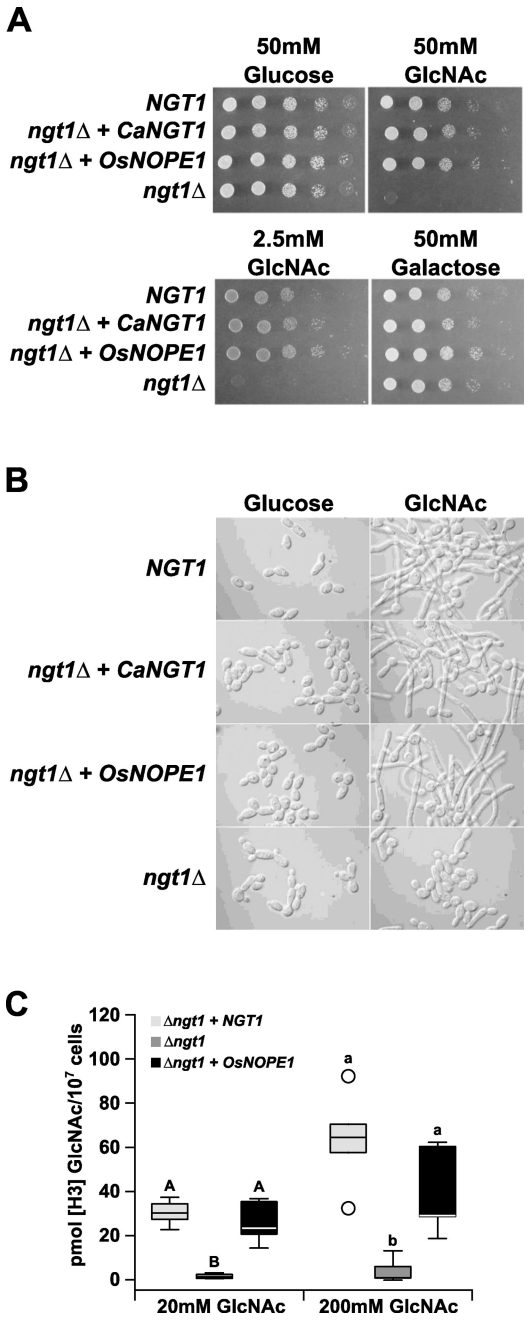

Figure 2. Rice NOPE1 mediates GlcNAc transport in *C. albicans*

A, Ten-fold cell dilution series of *C. albicans* strains spotted onto plates with indicated sugar. **B**, *C. albicans* strains grown overnight in glucose containing medium and resuspended in fresh medium containing either 50 mM glucose or 50 mM GlcNAc. **C,** [H^3]GlcNAc uptake in *C. albicans* strains at 20 mM and 200 mM GlcNAc. GlcNAc, N-acetylglucosamine. Boxes show 1st quartile, median and 3rd quartile. Whiskers extend to the most extreme points within 1.5× box length; outlying values beyond this range are shown as unfilled circles. Means groups were calculated *post hoc* independently for the two GlcNAc treatments and

are indicated by letters ($p < 0.05$). For description of strains see Supplemental Information Table S5.

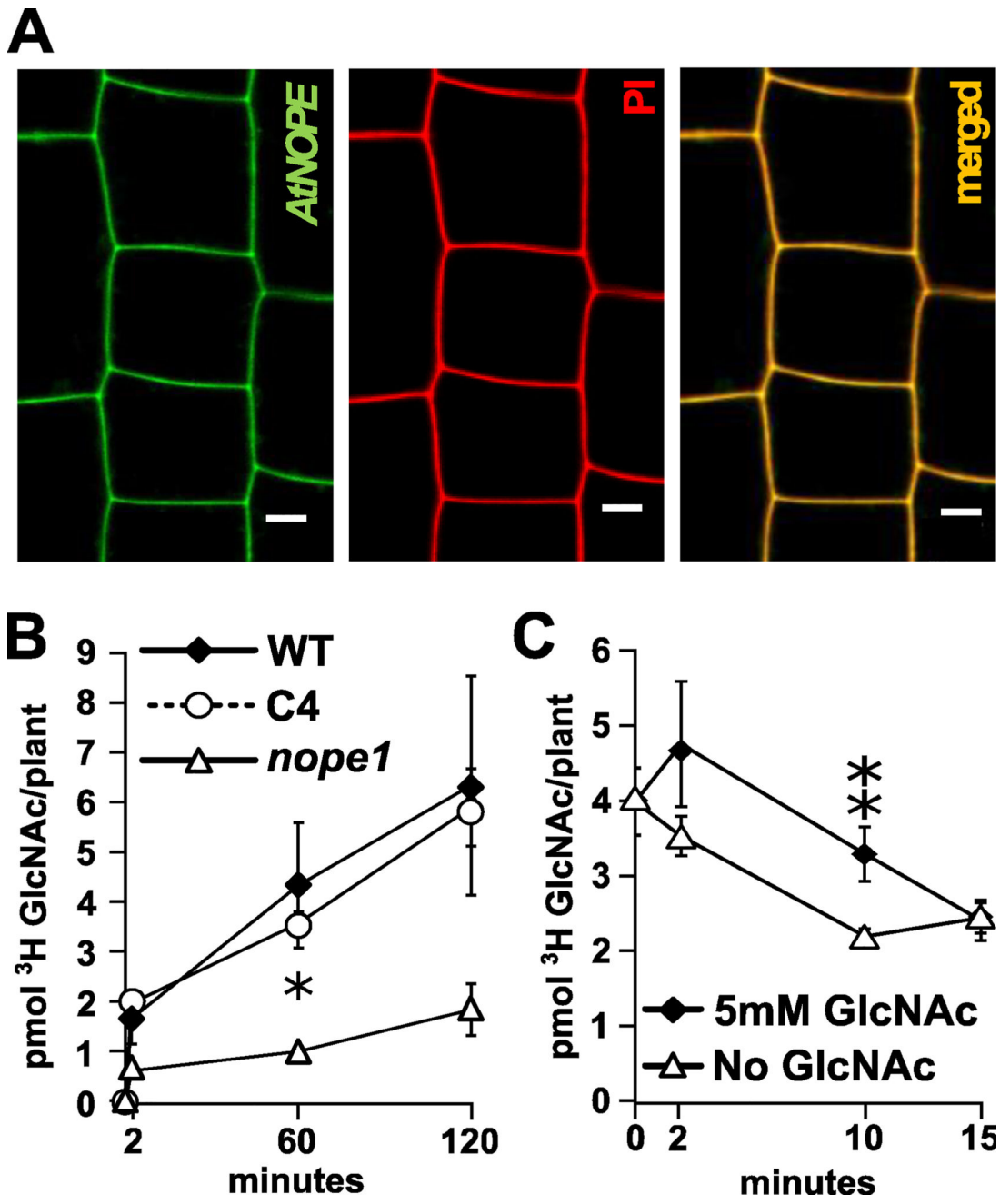

Figure 3. NOPE1 mediates GlcNAc transport in rice and *Arabidopsis*

A, Laser scanning confocal microscopy of *A. thaliana* roots expressing Ubq_{prom}*::YPF::AtNOPE1*. Three independent lines were analyzed to determine reproducible localization patterns, here shown for line *At4731y-4* with the YFP-AtNOPE1 signal shown in green (left). Corresponding cells stained with Propidium Iodide (PI) shown in red (centre). Overlay (yellow, right). Scale bar: 5 μm. **B,** Time course of [^{3}H]GlcNAc uptake in roots of O*snope1*, wild type and genetically complemented mutant line C4. Means and SEs of three biologically independent experiments are shown (*$P \leq 0.05$). Please note that the 0.5min value corresponds to unspecific adsorption of medium to the protoplasts. **C,** Time

course of [^{3}H]GlcNAc export activity of wild type rice roots at 0 and 5mM (50×) GlcNAc external concentration. Means and SEs of three biological replicates are shown. (**$p \leq 0.01$).

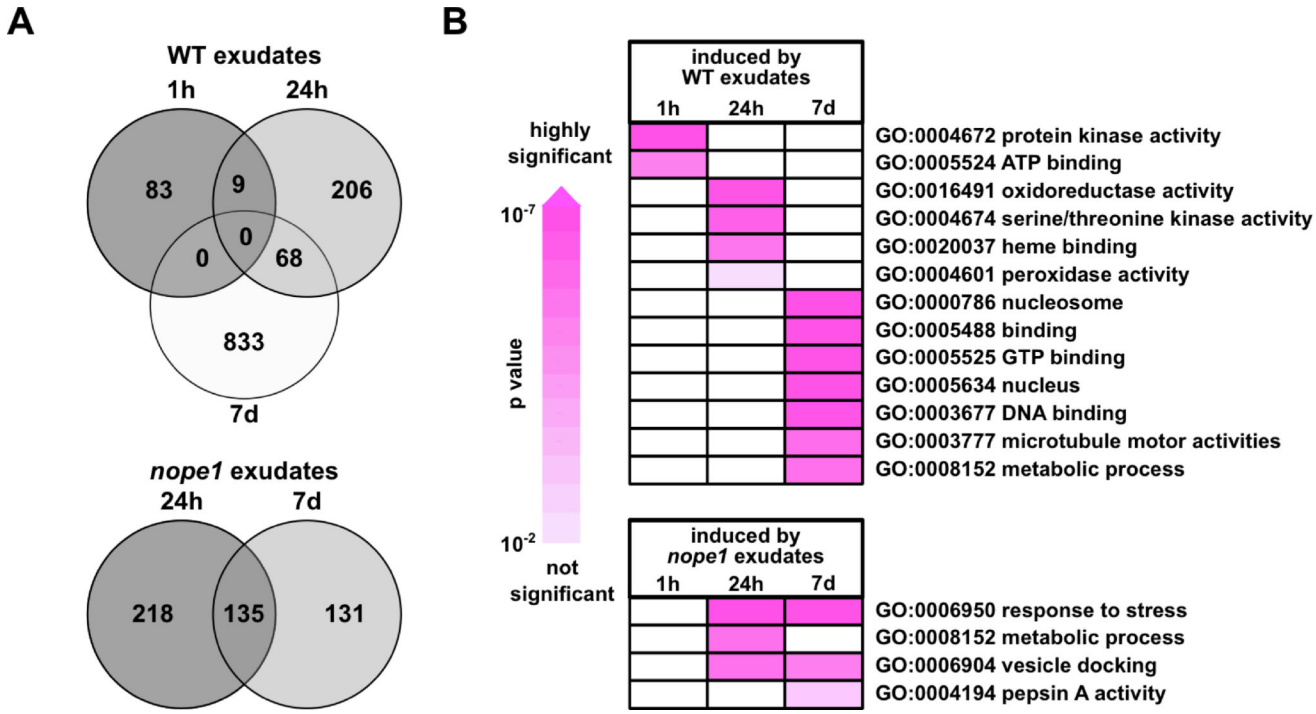

Figure 4. ***R. irregularis*** **transcriptional response to root exudates from rice wild type and** ***Osnope1*** **mutant plants**

A, Venn diagrams indicating number of significantly induced fungal genes ($P \leq 0.05$, one way ANOVA) in response to treatment with exudates from wild type relative to *Osnope* (top) and *Osnope* relative to wild type (bottom). **B,** Time-resolved Gene Ontology analysis for Biological Process terms ($p \leq 0.01$) for fungal genes induced when treated with root exudates from wild type (top) or from *Osnope1* (bottom). The colour code indicates the significance of gene enrichment (p-value).